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Purification and characterization of a (R)-2,3-butanediol dehydrogenase from Saccharomyces cerevisiae.
AU Heidlas J; Tressl R
CS Technische Universitat Berlin, Fachbereich Lebensmitteltechnologie und Biotechnologie.
SO ARCHIVES OF MICROBIOLOGY, (1990) 154 (3) 267-73.
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Molecular characterization of the Pseudomonas putida 2,3-butanediol catabolic pathway.
AU Huang M; Oppermann F B; Steinbuchel A
CS Department of Biology, Scihuan Normal University, Chengdu, China.
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Molecular characterization of the *Pseudomonas putida* 2,3-butanediol catabolic pathway

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Abstract: The 2,3-butanediol dehydrogenase and the acetoin-cleaving system were simultaneously induced in *Pseudomonas putida* PpG2 during growth on 2,3-butanediol and on acetoin. Hybridization with a DNA probe covering the genes for the E1 subunits of the *Alcaligenes eutrophus* acetoin cleaving system and nucleotide sequence analysis identified *acoA* (975 bp), *acoB* (1020 bp), *acoC* (1110 bp), *acoX* (1053 bp) and *adh* (1086 bp) in a 6.3-kb genomic region. The amino acid sequences deduced from *acoA*, *acoB*, and *acoC* for E1α (M_r 34639), E1β (M_r 37268), and E2 (M_r 39613) of the *P. putida* acetoin cleaving system exhibited striking similarities to those of the corresponding components of the *A. eutrophus* acetoin cleaving system and of the acetoin dehydrogenase enzyme system of *Pelobacter carbinolicus* and other bacteria. Strong sequence similarities of the *adh* translational product (2,3-butanediol dehydrogenase, M_r 38361) were obtained to various alcohol dehydrogenases belonging to the zinc- and NAD(P)-dependent long-chain (group I) alcohol dehydrogenases. Expression of the *P. putida* ADH in *Escherichia coli* was demonstrated. The *aco* genes and *adh* constitute presumably one single operon which encodes all enzymes required for the conversion of 2,3-butanediol to central metabolites.

Key words: 2,3-Butanediol dehydrogenase; Acetoin-cleaving system; Acetoin dehydrogenase enzyme system; *Pseudomonas putida*; *Alcaligenes eutrophus*; *Pelobacter carbinolicus*

Introduction

The key reaction of the fermentative breakdown of acetoin (3-hydroxy-2-butanone) in the strictly anaerobic bacteria *Pelobacter carbinolicus* and *Clostridium magnum* is the thiamine PP_i (TPP)-, coenzyme A-, and NAD-dependent cleavage of acetoin into acetaldehyde and acetyl coenzyme A, which is catalysed by the acetoin dehydrogenase enzyme system [1–3]. Utilization of the reduced acetoin-derivative 2,3-butanediol requires in addition 2,3-butanediol dehydrogenase which feeds the substrate to the acetoin dehydrogenase enzyme system. The structural genes of the acetoin dehydrogenase enzyme system *acoA* (encoding the α subunit of the TPP-dependent acetoin dehydrogenase, E1α), *acoB* (encoding E1β), *acoC* (encoding dihydrolipoamide acetyltransferase, E2), and *acoL* (encoding dihydrolipoamide dehydrogenase, E3) from both *P.*

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carbinolicus and *C. magnum* were found to be clustered in co-linear orientation in the genome [2,3]. In addition to the structural genes of the acetoin dehydrogenase enzyme system, *acoS* (encoding the *P. carbinolicus* lipoate synthase), *acoX* (encoding a *C. magnum* protein of unknown function), and *acoR* (encoding a putative regulatory protein) were localized within the gene cluster of the respective bacterium. A similar organization was recently also reported for the genes encoding the acetoin catabolic system of *Klebsiella pneumoniae* [4].

From the great variety of strictly respiratory acetoin-degrading bacteria, as yet only the molecular genetics of the acetoin catabolism of *Alcaligenes eutrophus* have been examined in detail [5]. The gene products and the molecular organization of the *A. eutrophus aco* operon (*acoXABC*) encoding the *A. eutrophus* acetoin cleaving system resemble in some aspects those of the acetoin dehydrogenase enzyme system of the anaerobic bacteria mentioned above. In contrast to the latter, *acoL* is absent in the *aco* locus of *A. eutrophus*, and the participation of a dihydrolipoamide dehydrogenase (E3) in the acetoin cleaving system remained obscure [6]. *Pseudomonas putida*, which in contrast to *A. eutrophus*, can also use 2,3-butanediol as sole carbon source in addition to acetoin [7], is unique since this bacterium possesses three different dihydrolipoamide dehydrogenases, of which the function of the third isoenzyme (LPD-3) is unknown [8]. As LPD-3 exhibited significant similarities to both E3 components of the acetoin dehydrogenase enzyme systems of *P. carbinolicus* [2] and *C. magnum* [3], this bacterium was chosen to investigate its acetoin-catabolic system with particular consideration to the participation of an E3 component.

Materials and Methods

Bacteria and media

Pseudomonas putida PpG2 (ATCC 23287, wild-type, kindly provided by J.R. Sokatch) and *Escherichia coli* XL1-Blue (Stratagene, La Jolla, CA) were used in this study. *P. putida* was grown at 30°C, in mineral salts medium [9], supplemented with 0.2% (w/v) of 2,3-butanediol, acetoin, glucose or acetate as carbon source. *E. coli* was grown at 37°C in Luria-Bertani (LB) [10] medium. For enzymatic analysis the soluble cell fractions were used from cells, harvested in the late exponential growth phase, resuspended in 50 mM 2-(*N*-morpholino)propane sulfonic acid (pH 7.2), disrupted by ultrasonication, and centrifuged 50 min at 100 000 × *g*.

Isolation and manipulation of DNA

Total genomic DNA from *P. putida* was obtained as described [11]. Plasmid DNA was isolated from *E. coli* by using the alkaline lysis method [10]. Other DNA manipulations were essentially as described [10]. To construct partial libraries, genomic DNA of *P. putida* was digested with *Eco*RI, *Apa*I or *Cla*I. DNA fragments of desired length were recovered from 0.8% (w/v) agarose gels by using a Gene Clean kit (Bio 101, La Jolla, CA) and ligated with linearized vector pBluescript KS$^-$ (Stratagene, La Jolla, CA) to transform competent cells of *E. coli* [10]. Transformants were screened by using in situ hybridization [10] with a heterologous biotinylated DNA probe. DNA sequencing was done by the dideoxy-chain termination method [12] with alkaline denatured double-stranded plasmid DNA using a T7 polymerase sequencing kit (Pharmacia LKB Biotechnology, Uppsala, Sweden). Sequence data were analysed with the Genetic Computer Group (GCG) sequence analysis software package [13].

Assay and analysis of proteins

Activities of the enzyme components E1, E2, and E3 of the acetoin dehydrogenase enzyme system, and of 2,3-butanediol dehydrogenase were determined photometrically according to Oppermann et al. [1] and Steinbüchel and Schlegel [9], respectively. Protein was determined as described by Lowry et al. [14]. Proteins of cell extracts were separated by denaturating SDS-polyacrylamide gel electrophoresis [15]. Activity staining for E1 was done as described before [1].

Table 1
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Table 1
Expression of *P. putida* PpG2 2,3-butanediol dehydrogenase

Strain	Plasmid	Cultivation	Specific activity (U(mg protein)$^{-1}$)
P. putida PpG2	–	MM + 2,3-butanediol	0.045
P. putida PpG2	–	MM + acetoin	0.04
P. putida PpG2	–	MM + glucose	< 0.001
E. coli XL1Blue	pBluescriptKS	LB + IPTG	< 0.001
E. coli XL1Blue	pKS::EA24	LB + IPTG	0.087
E. coli XL1Blue	pKS::EA24	LB + Glucose	< 0.001

P. putida was grown in mineral medium (MM) in the presence of 0.2% (w/v) of 2,3-butanediol, acetoin or glucose; cells of *E. coli* were cultivated in 300-ml Erlenmeyer flasks containing 30 ml LB with either 1.0 mM isopropyl-β-D-thiogalactopyranoside (IPTG) or 1.0% (w/v) glucose.

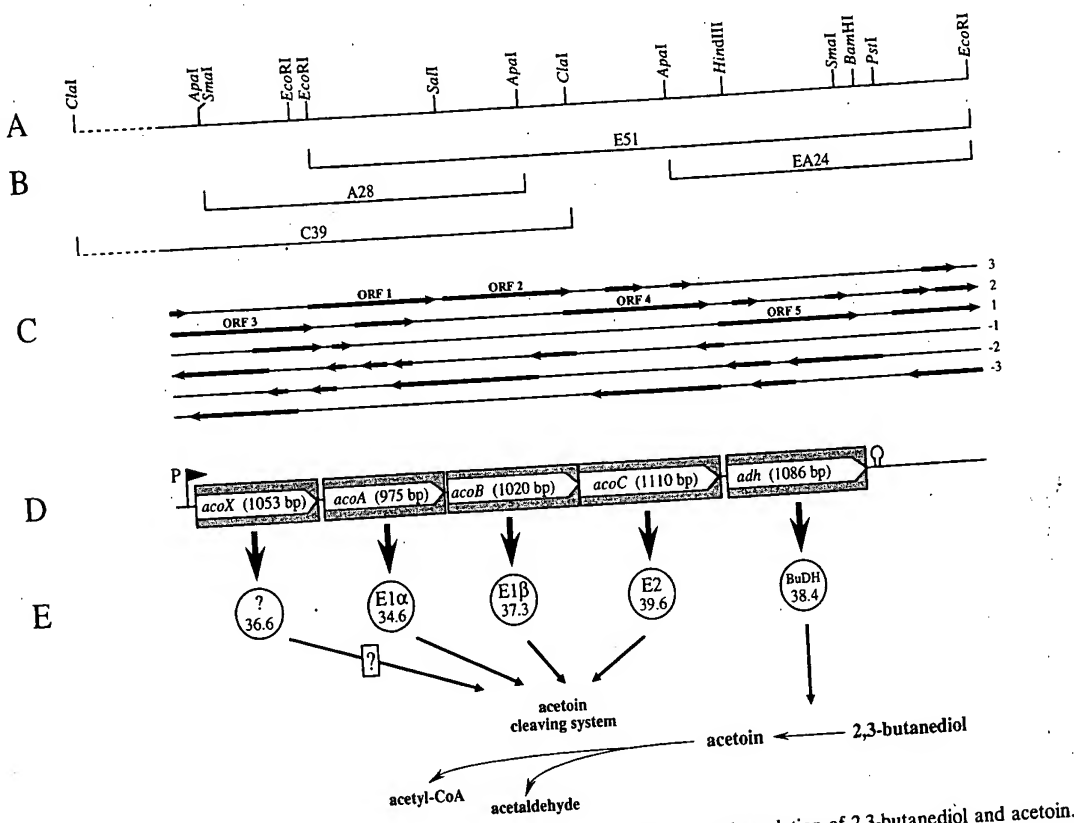


Fig. 1. Molecular organization of the *P. putida* PpG2 *aco* gene cluster involved in the degradation of 2,3-butanediol and acetoin. (A) Relevant restriction sites of the analysed region. (B) Relevant fragments analysed in this study. (C) ORFs detected in the sequenced region. ORFs comprising more than 150 bp are symbolized by arrows. (D) Structural genes of the *aco* gene cluster. The position of the putative promotor (P) and of the putative transcriptional termination signal (stem–loop) are indicated. (E) Putative 2,3-butanediol catabolic pathway and participation of the gene products. Molecular masses, which were calculated for the deduced gene products, are provided inside the circles in kDa.

Table 2

Similarities of the amino acid (aa) sequences deduced from the *P. putida* PpG2 *aco* genes to the respective *aco* gene products of *A. eutrophus* [5], *P. carbinolicus* [2], *K. pneumoniae* [4] and *C. magnum* [3]

Aa sequence deduced from *P. putida* gene	Percentage of amino acid identity to the respective *aco* gene product from			
	A. eutrophus	*P. carbinolicus*	*K. pneumoniae*	*C. magnum*
acoA	63.6	64.2	59.1	48.9
acoB	63.2	52.7	51.6	41.3
acoC	47.5	29.4	25.9	21.4
acoX	42.4	Not present	Not present	30.8

Values were calculated for complete sequence overlap by the program GAP [13].

Results and Discussion

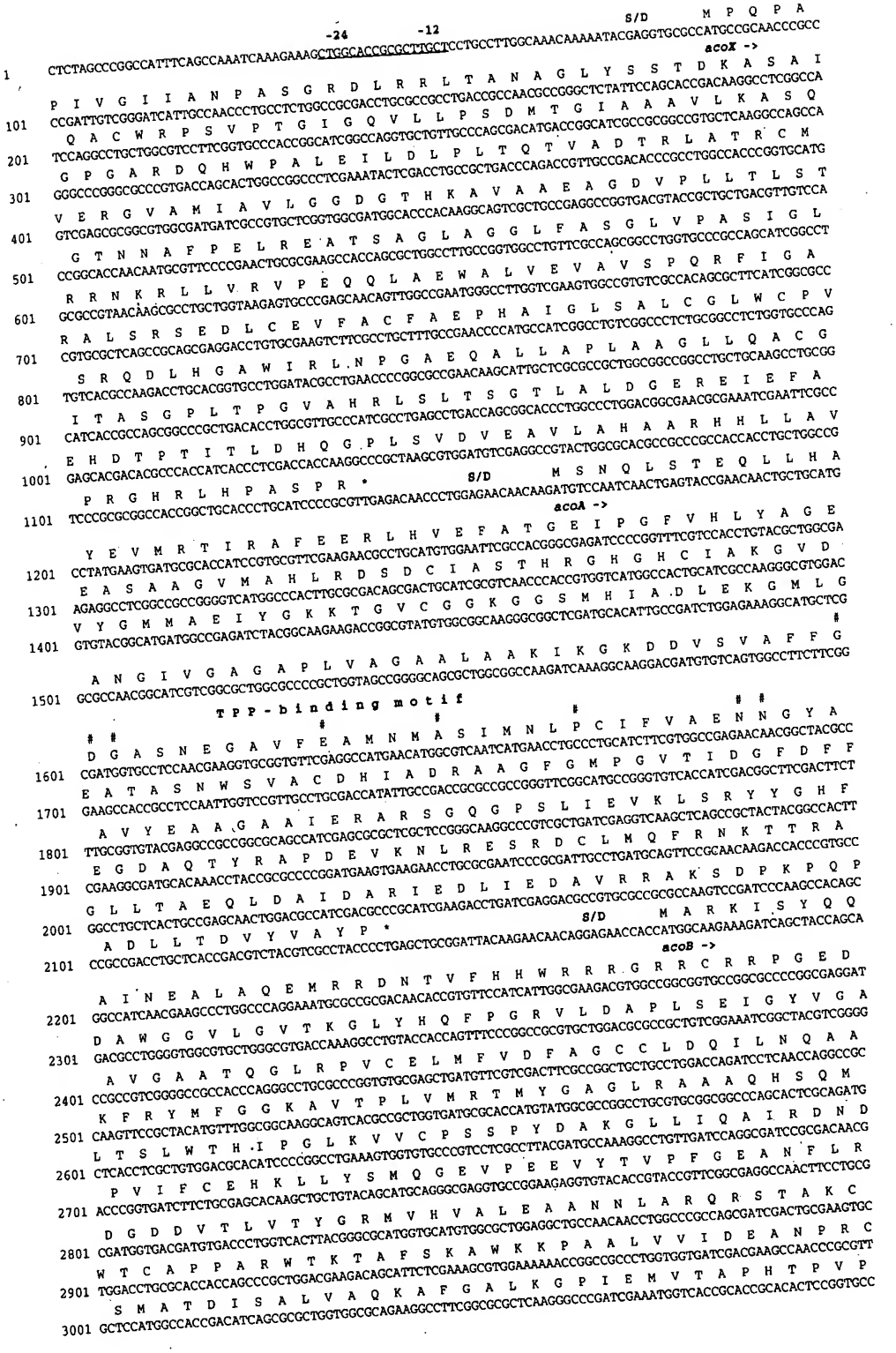
Induction of the 2,3-butanediol-catabolic enzymes

To investigate the presence of the components of the acetoin dehydrogenase enzyme system after growth on 2,3-butanediol and on acetoin, cells of *P. putida* were grown on different substrates and assayed for their respective enzyme activities. Growth on acetoin and on 2,3-butanediol was fairly effective with doubling times (t_d) of 2.2 h and 2.4 h, respectively, whereas on the non-acetoinogenic substrates, glucose and acetate, t_d values were obtained of 2.2 and 2.3 h, respectively. TPP-dependent acetoin dehydrogenase (E1) was only detected in the soluble cell fractions after growth on acetoin (0.070 U (mg protein)$^{-1}$) or on 2,3-butanediol (0.072 U mg^{-1}), whereas no activity was measurable after growth on either glucose or acetate. In accordance with that, one protein band with an R_f-value of 0.12 occurred during activity staining for E1 in the two protein patterns from acetoin- or 2,3-butanediol-grown cells, whereas none occurred with proteins derived from glucose- or acetate-grown cells. Expression of 2,3-butanediol dehydrogenase exhibited a similar regulatory pattern (Table 1). In contrast, dihydrolipoamide acetyltransferase (E2) and dihydrolipoamide dehydrogenase (E3) were measured after growth on either substrate (U mg^{-1} after growth on acetoin: E2, 0.15, E3, 0.083; 2,3-butanediol: E2, 0.15, E3, 0.064; glucose: E2, 0.30, E3, 0.085; acetate: E2, 0.21, E3, 0.23), which can be explained by the presence of the isoenzymes for E2 and E3 from the pyruvate and/or 2-oxoglutarate dehydrogenase complex. This indicated a close regulatory linkage between the expression of the *P. putida* acetoin catabolic system and 2,3-butanediol dehydrogenase which is different from the situation previously found in *A. eutrophus* [16]: although a multifunctional alcohol dehydrogenase able to oxidize 2,3-butanediol [9] is encoded in the *A. eutrophus* genome, the wild-type cannot grow on 2,3-butanediol, since this fermentative enzyme is only expressed under conditions of restricted oxygen supply.

Identification and characterization of the structural genes encoding the acetoin cleaving system

Southern hybridization analysis of totally digested *P. putida* PpG2 genomic DNA with fragment ES25, which covered the structural genes *acoA* and *acoB* encoding E1α and E1β, respec-

Fig. 2. Nucleotide sequence of the *P. putida* PpG2 *aco* gene cluster. Amino acids deduced from the nucleotide sequence are specified by standard one-letter abbreviations. The position of a putative $-24/-12$ promotor and of putative ribosomal binding sites (S/D) are indicated. The position of a putative stem–loop structure is marked by inverted arrows. Conserved amino acid residues of the putative TPP-binding region of the deduced *acoA* gene product, and conserved glycine residues flanking the putative lipoylation site of the *acoC* gene product are marked by the symbol #; the position of the lysine in the deduced *acoC* gene product, which is presumably lipoylated, is indicated by the symbol *. The nucleotide and amino acid sequences have been submitted to the Genome Sequence Database at the Los Alamos National Laboratory under accession no. L35343.


-24
-12
S/D
acoX ->
acoA ->
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TPP-binding motif
S/D
acoB ->

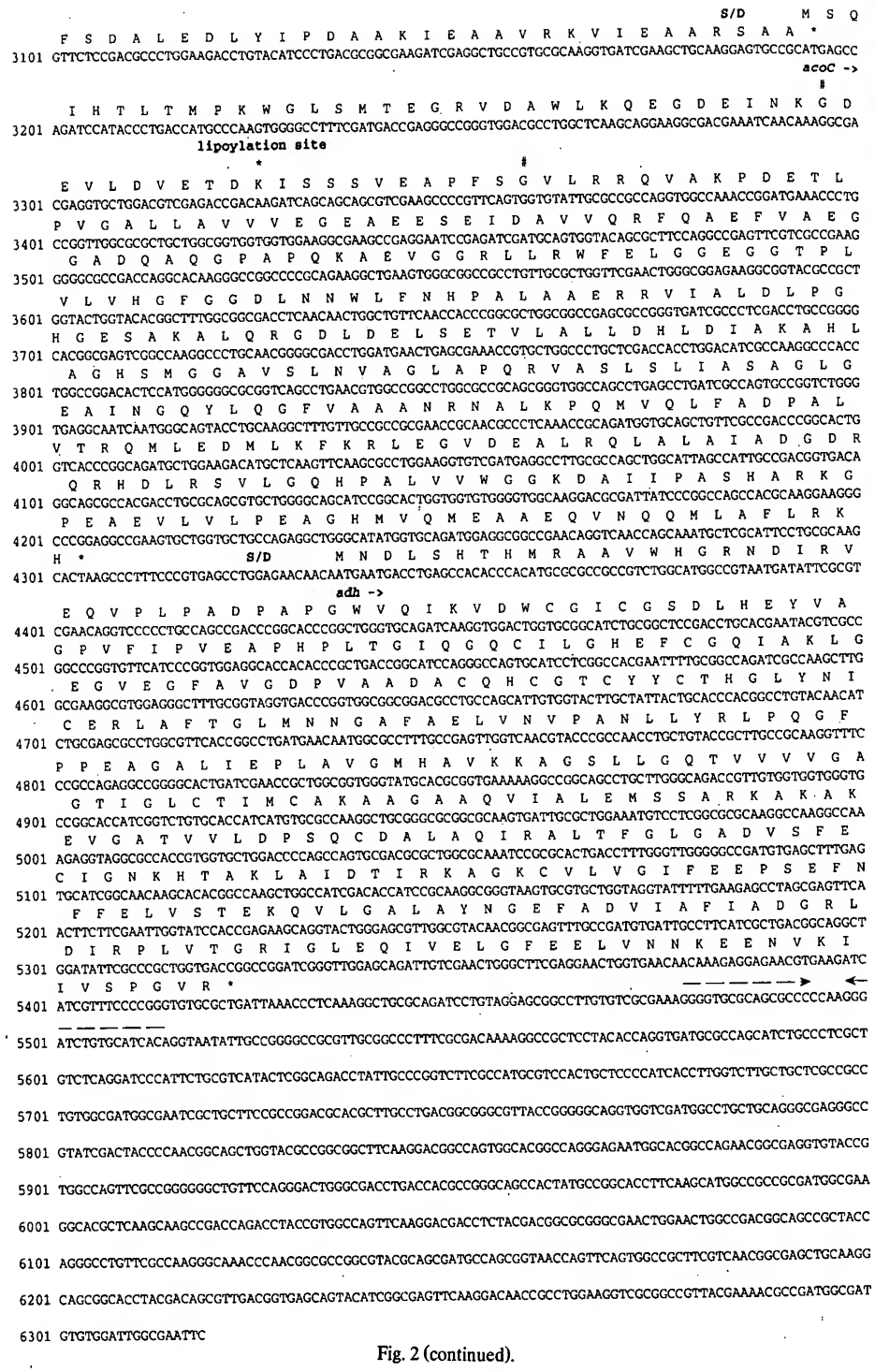

Fig. 2 (continued).

tively, of the *A. eutrophus* acetoin cleaving system [5], revealed clear signals corresponding to a 5.1-kb *Eco*RI, a 2.8-kb *Apa*I and a 3.9-kb *Cla*I fragment. In situ hybridization with the corresponding partial libraries in *E. coli* identified four *Eco*RI, five *Apa*I and two *Cla*I clones. Se-

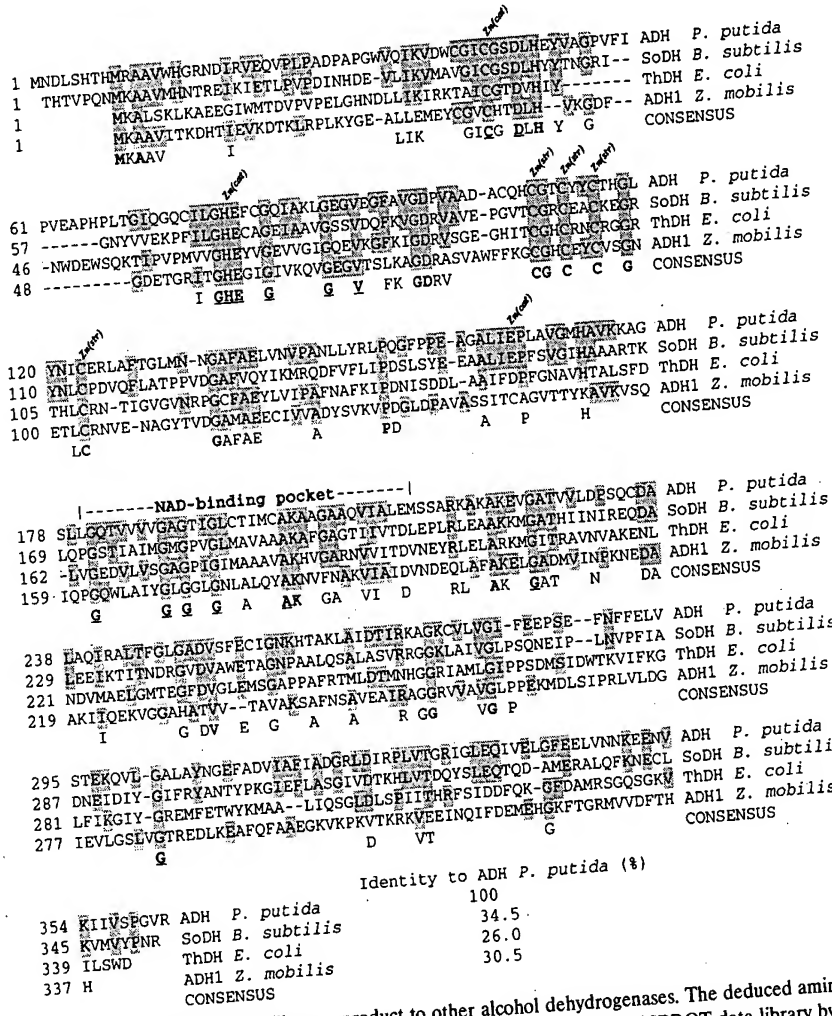

Fig. 3. Homology of the *P. putida* PpG2 *adh* gene product to other alcohol dehydrogenases. The deduced amino acid sequence of the *P. putida adh* gene product was compared with the polypeptides stored in the SWISSPROT data library by using the program FASTA [13]. Microbial amino acid sequences, which gave the best sequence overlaps with the *P. putida* protein, were aligned by using the program MULTALIGN [13]. Amino acids are specified by standard one-letter abbreviations, and the numbers indicate the position of the respective residue within the protein. Regions with identity to the *P. putida adh* gene product shown in the upper line are shaded. Amino acids, which are present in at least three of the four proteins, are labelled as CONSENSUS; amino acids, which are conserved in all four sequences, are written in bold; the strictly conserved residues of microbial group I alcohol dehydrogenases [19] are underlined. The putative ligands of the catalytic zinc [Zn(cat)] and of the structural zinc atom [Zn(str)] as well as the putative cofactor-binding site of the *P. putida* protein are indicated above the aligned sequences. Calculation of the percentages of identity of the amino acid sequences to the *P. putida* polypeptide was done for a complete sequence overlap with the program GAP [13]. Amino acid sequences from the proteins of *B. subtilis*, *E. coli* and *Z. mobilis* were fetched from the SWISSPROT data library: SoDH, sorbitol dehydrogenase (accession no. Q06004); ThDH, threonine dehydrogenase (accession no. P07913); ADH1, alcohol dehydrogenase I (accession no. P20368).

quence analysis of the corresponding fragments (E51, A28, and C39 in Fig. 1) revealed 6318 bp of the *P. putida* genome exhibiting a G + C content of 65.1 mol%, which corresponded to the G + C content of 62.5 mol% of the genome [7]. Among 32 open reading frames (ORFs) with a minimum length of 150 bp, five (ORF1, ORF2, ORF3, ORF4, and ORF5 in Fig. 1) were identified as structural genes according to the following characteristics: (i) agreement with the *P. putida* codon preference; (ii) presence of reliable Shine/Dalgarno sequences; (iii) similarity to gene products stored in data libraries.

Due to clear homologies to the corresponding enzyme components of the acetoin catabolic systems of *A. eutrophus*, *P. carbinolicus*, *C. magnum*, and *K. pneumoniae*, ORF1 and ORF2 were identified as genes *acoA* and *acoB*, encoding the α and β subunit of the acetoin dehydrogenase (E1), whereas ORF4 corresponded to *acoC* encoding the E2 component (Fig. 1, Table 2). A TPP-binding motif [17] was identified in the translational product of *acoA* (Fig. 2). In the translational product of *acoC*, the consensus sequence of a lipoyl-binding site [18] was localized (Fig. 2). ORF3, which was located upstream of *acoA*, exhibited strong homologies to both *acoX* translational products (Table 2), which were previously identified in the *aco* gene clusters of *C. magnum* [3] and of *A. eutrophus* [5], and was therefore referred to as *acoX*. As no further significant similarity to any other gene product was obtained, and as no clear evidence for the presence of catalytic or structural motifs was obtained from the consensus sequence of the *acoX* gene products, the function of *acoX* in the acetoin catabolism of *A. eutrophus*, *C. magnum* and *P. putida* is obscure. Closely upstream of *acoX* a motif was located which matched the enterobacterial σ^{54} promotor consensus sequence [5] (Fig. 2). The program STEMLOOP [13] predicted a region of dyad symmetry downstream of ORF5 (Fig. 2), suggesting that there is a possible mRNA structure with a free energy of stem–loop formation of 53.6 kJ which might serve as a transcriptional terminator. The nucleotide sequence data provided no evidence for the presence of a structural gene encoding a dihydrolipoamide dehydrogenase, which is similar to the situation previously reported for *A. eutrophus* [5]. Thus, the participation of an E3 component in the acetoin cleaving system of strictly respiratory bacteria remains obscure.

Identification and characterization of adh encoding 2,3-butanediol dehydrogenase

Downstream of and co-linear to *acoC*, ORF5 was identified encoding a 362-amino acid polypeptide which exhibited a high degree of homology to various alcohol dehydrogenases (ADHs) belonging to the NAD(P)- and zinc-dependent long-chain (group I) ADHs [19]. It was therefore concluded that ORF5 represents an ADH structural gene. The alignment of the primary structure deduced from ORF5 with representative ADH sequences matched all 14 strictly conserved residues of microbial group I ADHs [19] including the motifs around two of the three catalytic zinc ligands (Cys_{46} and His_{79}) at the N-terminal part, and the NAD-binding pocket at the central part of the polypeptide (Fig. 3). In addition, the binding motif for a second zinc was identified (Cys_{109} to Cys_{123}), which is known to bind the non-catalytic or structural zinc in most group I ADHs [19]. Interestingly, the putative third ligand of the catalytic zinc in *P. putida* ADH was found to be a negatively charged residue (Glu_{165}) which is also present in ADHs catalysing the oxidation of secondary alcohols (e.g. sorbitol dehydrogenase from *Bacillus subtilis*, threonine dehydrogenase from *E. coli*; Fig. 3). In almost all other ADHs, which preferentially accept primary alcohols, this position is occupied by a cystein residue (e.g. alcohol dehydrogenase I from *Zymomonas mobilis*; Fig. 3). These sequence data correspond with a catabolic function of the *P. putida adh* gene product in feeding 2,3-butanediol to the acetoin cleaving system. In addition, the absence of promotor-like structures upstream of *adh*, *acoC*, *acoB*, and *acoA*, the presence of a reliable promotor region upstream of *acoX*, the concomitant synthesis of the acetoin-cleaving system and of the 2,3-butanediol dehydrogenase during growth on 2,3-butanediol and on acetoin provided evidence that *adh* belongs to the same operon as the four *aco* genes.

Heterologous expression of ADH in E. coli

Since fragment EA24 harbours the entire *adh*gene (Fig. 1), it was chosen for expression of ADH in *E. coli*. Crude cell extracts of IPTG-induced *E. coli* (pKS::EA24) exhibited significant 2,3-butanediol dehydrogenase activity (Table 1). No enzyme activity was detected in cells grown on glucose or in cells of *E. coli* (pBluescriptKS). In SDS-polyacrylamide gels, two distinct protein bands with estimated M_r values of 38000 and 47000 were visible in the protein pattern of IPTG-induced cells of *E. coli* (pKS::EA24), which did not appear in glucose-repressed *E. coli* (pKS::EA24) or in *E. coli* harbouring only the vector. The protein band corresponding to the smaller protein also appeared in the protein pattern of *P. putida* PpG2 grown on acetoin or 2,3-butanediol (Fig. 4), and its size corresponded to the calculated value of the *adh* gene product (M_r 38361).

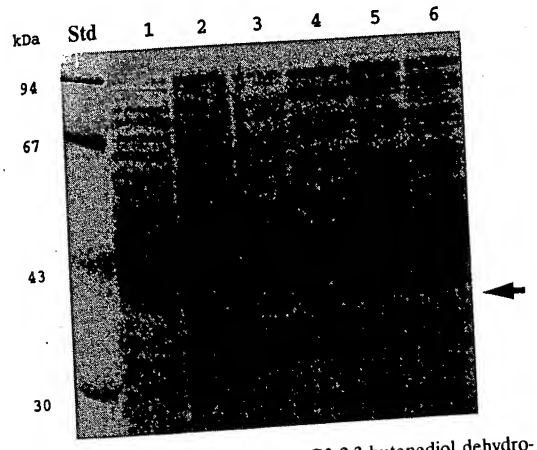

Fig. 4. Expression of *P. putida* PpG2 2,3-butanediol dehydrogenase in *E. coli*. Soluble extracts from *P. putida* PpG2 and from recombinant strains of *E. coli* XL1-Blue were obtained from cells harvested in the late exponential growth phases. Proteins were separated in an SDS-11.5% (w/v) polyacryamide gel and stained with Serva Blue R. Molecular masses of standard proteins (lane Std) are given. The position of a protein band in lane 4 corresponding to the predicted M_r of *P. putida* 2,3-butanediol dehydrogenase is marked by an arrow. Lanes: 1, 28 μg protein of the soluble cell fraction from *P. putida* grown on glucose; 2, 38 μg of protein of the soluble cell fraction from *P. putida* grown on acetoin; 3, 29 μg of protein of the soluble cell fraction from *P. putida* grown on 2,3-butanediol; 4, 25 μg of protein of the soluble cell fraction from *E. coli* XL1-Blue(pKS::EA24) grown in the presence of 1.0 mM IPTG; 5, 28 μg of protein of the soluble cell fraction from *E. coli* XL1-Blue(pKS::EA24) grown in the presence of 1% (w/v) glucose; 6, 31 μg of protein of the soluble cell fraction from *E. coli* XL1-Blue(pBluescriptKS$^-$).

Conclusions

The data presented here provide evidence that in *P. putida* PpG2 2,3-butanediol is metabolized (i) by oxidation to acetoin, which is catalysed by the 2,3-butanediol dehydrogenase, and (ii) by subsequent oxidative cleavage of acetoin to acetaldehyde and acetyl-CoA, which is catalysed by the acetoin cleaving system (Fig. 1E). The molecular organization of the genes involved in the second step resembled that previously reported for the respiratory acetoin-degrading *A. eutrophus* [5]: the structural genes encoding the enzyme components E1 and E2 of the acetoin cleaving system were closely clustered in the order *acoA*, *acoB* and *acoC*, and they were preceded by *acoX*, encoding a gene product of unknown function. As *adh* presumably belongs to the same operon in addition to the *aco* genes, all enzymes required for the conversion of 2,3-butanediol to central metabolites are expressed from one transcriptional unit.

Acknowledgements

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(FILE 'HOME' ENTERED AT 10:27:03 ON 06 MAY 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 10:27:08 ON 06 MAY 2003

L1 157 S BUTANEDIOL (1N) DEHYDROGENASE
L2 3 S L1 AND PICHIA
L3 3 DUP REM L2 (0 DUPLICATES REMOVED)
L4 94 S L1 AND ACETOIN
L5 9 S L1 AND (ACETOIN (2N) R)
L6 6 DUP REM L5 (3 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 10:29:31 ON 06 MAY 2003

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 10:33:52 ON 06 MAY 2003

L7 3 S L4 AND BUTANEDIONE
L8 3 DUP REM L7 (0 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 10:36:59 ON 06 MAY 2003

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 10:40:13 ON 06 MAY 2003

L9 14 S R (3N) L1
L10 8 DUP REM L9 (6 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 10:42:02 ON 06 MAY 2003

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 10:42:46 ON 06 MAY 2003

L11 121 S L1 AND (NAD OR NADH OR ACETOIN OR BUTANEDIONE)
L12 66 S L1 AND (NAD OR NADH)
L13 42 S L12 AND ACETOIN
L14 8 S L13 AND R
L15 5 DUP REM L14 (3 DUPLICATES REMOVED)
L16 24 S L1 AND R
L17 13 DUP REM L16 (11 DUPLICATES REMOVED)

=>

(FILE 'HOME' ENTERED AT 08:50:10 ON 08 MAY 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 08:50:16 ON 08 MAY 2003

L1 140 S ACETOIN (2N) REDUCTASE
L2 2 S L1 AND R
L3 1 DUP REM L2 (1 DUPLICATE REMOVED)
L4 3 S L1 AND PICHIA
L5 1 DUP REM L4 (2 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 08:51:33 ON 08 MAY 2003
L6 0 S L1 AND BUTANEDIOL

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 08:52:04 ON 08 MAY 2003

L7 56 S L1 AND BUTANEDIOL
L8 33 DUP REM L7 (23 DUPLICATES REMOVED)
L9 0 S L8 AND D HIS

L10 ANSWER 6 OF 8 MEDLINE DUPLICATE 2
AN 91024485 MEDLINE
DN 91024485 PubMed ID: 2222122
TI Purification and characterization of a (**R**)-2,3-**butanediol dehydrogenase** from Saccharomyces cerevisiae.
AU Heidlas J; Tressl R
CS Technische Universitat Berlin, Fachbereich Lebensmitteltechnologie und Biotechnologie.
SO ARCHIVES OF MICROBIOLOGY, (1990) 154 (3) 267-73.
Journal code: 0410427. ISSN: 0302-8933.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199011
ED Entered STN: 19910117
Last Updated on STN: 19970203
Entered Medline: 19901121
AB A NAD-dependent (**R**)-2,3-**butanediol dehydrogenase** (EC 1.1.1.4), selectively catalyzing the oxidation at the (R)-center of 2,3-butanediol irrespective of the absolute configuration of the other carbinol center, was isolated from cell extracts of the yeast Saccharomyces cerevisiae. Purification was achieved by means of streptomycin sulfate treatment, Sephadex G-25 filtration, DEAE-Sepharose CL-6B chromatography, affinity chromatography on Matrex Gel Blue A and Superose 6 prep grade chromatography leading to a 70-fold enrichment of the specific activity with 44% yield. Analysis of chiral products was carried out by gas chromatographic methods via pre-chromatographic derivatization and resolution of corresponding diastereomeric derivatives. The enzyme was capable to reduce irreversibly diacetyl (2,3-butanediol) to (R)-acetoin (3-hydroxy-2-butanone) and in a subsequent reaction reversibly to (R,R)-2,3-butanediol using NADH as coenzyme. 1-Hydroxy-2-ketones and C5-acyloins were also accepted as substrates, whereas the enzyme was inactive towards the reduction of acetone and dihydroxyacetone. The relative molecular mass (Mr) of the enzyme was estimated as 140,000 by means of gel filtration. On SDS-polyacrylamide gel the protein decomposed into 4 (identical) subunits of Mr 35,000. Optimum pH was 6.7 for the reduction of acetoin to 2,3-butanediol and 7.2 for the reverse reaction.